REMARKS/ARGUMENTS

Status of the Claims

Claims 37-65 are pending and presented for examination. Claim 37, 45, 46, and 62 are herein amended. Claims 40, 58 and 59 are canceled without prejudice.

Claims 37-65 stand rejected under the judicially created doctrine of double patenting in view of U.S. Patent Nos. 5,504,117 and 5,693,676.

Claims 37-65 stand rejected as being unpatentable under 35 U.S.C. §103(a) over Loder *et al.*, (Gut, 1993: Abstract P22/64), Loder *et al.* (Dis Colon Rectum Mtg. Abstracts, 1993: Abstract T96/S25) (collectively referred to herein as the "Loder Abstracts"), and Guillemot *et al.* (Disc. Colon Rectum, 1993, 372-276, "Guillemot"); and further in view of Jensen (British Medical Journal, 1986,1167-1169, "Jensen"), and Gallina (U.S. Patent No. 4,514,384, "Gallina").

Applicants respectfully request reconsideration of the above grounds for rejection in view of the following remarks.

Support for the Amendments to the Claims

Claim 1 has been amended to recite "A method for ameliorating pain associated with an anal disorder in a human selected from one or more of the group consisting of anal fissure, anal ulcer, and hemorrhoidal disease" and to also recite " wherein said method the pain is ameliorated." Support for this subject matter is found *inter alia* in the previous version of the claim.

Amendments to dependent claims 45 and 45 correspond to changes in their antecedent basis in claim 37.

Claim 62 has been amended to recite "A method of treating a human patient having pain associated with an anal fissure, anal ulcer, or hemorrhoid," and to also recite " wherein said method the pain is ameliorated." Support for this subject matter is found *inter alia* in the previous version of the claim.

RESPONSE TO THE REJECTION FOR OBVIOUSNESS-TYPE DOUBLE PATENTING

The present application is rejected under the judicially created doctrine of obviousness-type double patenting over U.S. Patent Nos. 5,504,117 and 5,693,676. In response, Applicants respectfully request that this rejection be held in abeyance until some claims are deemed to be in condition for allowance. At such time, Applicants intend to submit a suitable terminal disclaimer to obviate any maintained obviousness type double patenting rejection.

RESPONSE TO THE REJECTION UNDER 35 U.S.C. § 103(a)

Claims 37-65 stand rejected as allegedly obvious over the Loder Abstracts and Guillemot; and further in view of Jensen and Gallina. Without acquiescing to the position of the Examiner, and in order to facilitate examination of the instant application, Applicants first note that they have amended the base claims so that they no longer recite "levator spasm."

The Standard of Review

The requirements for a *prima facie* case of obviousness, are set forth in M.P.E.P. § 2143:

> [t]o establish a *prima facie* case of obviousness, *three* basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Here, the reasonable expectation of success posited in the Action fails on two separate grounds. First, the ability of nitroglycerin to lower resting anal pressures is not much predictive of its utility for treating anal fissure, anal ulcers, or hemorrhoids. Applicants acknowledge that the Loder Abstracts and Guillemot do show an effect of nitroglycerin in relaxing the internal anal sphincter as measured by lowering of anal pressures. However, the ability of their different

treatment regimens to lower anal pressures does not create a reasonable expectation of success with respect to the treatment of *anal fissures, anal ulcers, or hemorrhoidal disease.*

Guillemot treated subjects with terminal constipation, rather than any of the above conditions. Even with respect to terminal constipation, Guillemot did not describe that the condition was alleviated. Guillemot stated only that additional studies exploring the therapeutic potential of nitroglycerin were then currently underway. However, no subsequent studies appear to have been published. A search of the PubMed web site with the term "Guillemot" and either "constipation" or "fissure" turned about no additional references (see Exhibits 1 and 2).

The Loder Abstracts themselves do not provide much detail with respect to their study populations. However, Loder et al. published this work more completely (*see*, enclosed, Loder et al., *Br J. Surg.* 81:1386-9 (1994), "the 1994 Loder reference, " and, particularly, the footnote on page 1 of the 1994 Loder referencewhich states the relationship of the 1994 Loder reference to the work reported in the Loder Abstracts). The 1994 Loder reference did not show that the Loder et al. methods were effective in the one patient suffering from that disorder amongst the subjects in their preliminary study. Loder et al. state in the first sentence of the first full paragraph of the last column of the 1994 Loder reference:

> It is yet to be established whether the preparation used will be effective in the treatment of anal fissure: only one patient with fissure was included in this preliminary study and the mechanism of raised pressure in patients with this lesion remains obscure.

The 1994 Loder reference also observed that "*Although several substances decrease anal pressure, none has found clinical application.*" (see the second sentence of the Discussion at p. 1388). Indeed, Loder's own colleagues subsequently reported(*see*, enclosed, Watson SJ, Kamm MA, Nicholls RJ, Phillips RK *Br J Surg.* 83(6):771-5 (1996)) further on the effects of topical glyceryl trinitrate in the treatment of chronic anal fissure with not much, if any, indication of success with respect to either lowering resting anal pressures in patients with anal fissures (see Figure 1 of the Watson reference) or in actually healing fissures when compared to the natural healing rate of such fissures. Most anal fissures come and go (see Watson at p. 774, first sentence, second full paragraph of discussion). At three weeks, the fissure healing rate

among the patients was accepted the treatment was about 40% (slightly higher or lower depending on whether the patients who dropped out are counted). This rate is dramatically less successful that the 87% healing rate achieved by nonchemical treatment using sitz baths and bran (*See*, Jensen et al. (already of record and cited in the instant Action) or conservative medical management which results in a 42% healing rate (see, Gough, Br J Surg. 1983 Mar;70(3):175-6 (cited in the 1996 Loder reference, Abstract, enclosed). Indeed, Loder's colleagues suggested the following reasons for the failure of their treatment method:

> treatment with topical GTN (nitroglycerin) on a twice-daily regimen may not produce fissure healing through two possible mechanisms. These are, the development of tachyphylaxis to the nitric oxide donor or a shorter duration of action of topical GTN in fissure patients compared to controls.

Thus, at the time the instant application was filed, the Loder et al. and Guillemot et al. reports that topical nitroglycerin was effective in relaxing the internal anal sphincter and in reducing pressures within the internal anal canal would not have sustained a reasonable basis for further expecting success in treating *anal fissure, anal ulcer, or hemorrhoidal disease,* or more particularly, the *pain* associated with these conditions.

Further, with respect to pain, Applicants note that the two base claims have been amended as follows to recite:

> Claim 37: A method for ameliorating *pain* in a human associated with an anal disorder selected from one or more of the group consisting of anal fissure, anal ulcer, and hemorrhoidal disease, comprising administering an effective amount of an organic nitric oxide donor proximate to, or to, the affected area of the patient, and wherein said method the pain is ameliorated.

> Claim 62: A method of treating a human patient having *pain* associated with an anal fissure, anal ulcer, or hemorrhoid, comprising applying a composition comprising an effective amount of an organic compound which can release nitric oxide under physiological or anal disease treatment conditions and a physiologically acceptable carrier to an area proximate to, or to, the affected area of the patient, wherein said method the pain is ameliorated.

In contrast, Applicants note that Guillemot reported that in their method, nitroglycerin actually caused pain, a local anal burning pain, in several subjects. These subjects, while presumably constipated, were not even described as having especially sensitive lesions such as anal fissures within their anal canals (see Guillemot last two lines of first column on page 374). Thus, with respect to ameliorating pain in particular, the Loder Abstracts and/or Guillemot provide no reasonable expectation of success that their methods would be effective in treating pain associated with anal fissures, anal ulcers, or hemorrhoids.

In contrast, Applicants' invention relates to the application of a nitric oxide donor to the anal canal for the treatment of anal disorders in which the anal canal tissue is **injured and damaged**; and the patient is experiencing pain associated with the disorder. Applicants respectfully assert that a skilled artisan, knowing the details of Guillemot's pilot study, *i.e.*, that applying a nitric oxide donor to apparently intact anal canal tissue results in anal burning pain, would not be motivated to apply a nitric oxide donor to a patient that has damaged anal tissue and is already experiencing pain. In fact, Applicants respectfully submit that Guillemot's disclosure that the application of a nitric oxide donor to the anal canal of constipated patients, who otherwise have healthy anal tissue, induced an anal burning pain in the anal canal actually ***would make it seem very unlikely that*** applying a nitric oxide donor proximate to, or to, the affected tissue would ameliorate pain associated with the affected tissue. As such, Applicants respectfully submit the disclosure of Guillemot in combination with Loder Abstracts simply does not provide a reasonable expectation of success as required pursuant to MPEP § 2143.

Moreover, Applicants respectfully assert that common knowledge in the art, with respect to the topical use of a nitric oxide donor, at the time of the invention was made also ***teaches away*** from the application of a nitric oxide donor to broken or injured skin. As evidence, Applicants respectfully direct the Examiner's attention to Exhibit 3 which is an article that appeared in RN Journal titled "Making the Most of Topical Nitroglycerin." The objective of the article was to instruct medical professionals as to "how to use topical NTG (nitroglycerin, a nitric oxide donor) safely and effectively" (*see*, 1st col., 2nd para.). The article warns the medical professional to "keep the medication (the nitric oxide donor) away from skin folds, scar tissue, and burned or *irritated areas.*" [Emphasis Added] In contrast, Applicants have found

that applying a nitric oxide donor proximate to, or to, the affected tissue (*e.g.*, anal fissure) is effective in reducing the pain associated with the disorder.

In view of the above, Applicants respectfully assert that the common knowledge in the art strongly teaches away from applying a nitric oxide donor to irritated or broken tissue to treat pain. Thus, a skilled artisan would not have reasonably expected the proposed combination to be effective in reducing or treating the pain associated with anal fissures, anal ulcers, or hemorrhoids when the nitric oxide donor is actually to be applied proximate to, or to, the *affected* tissue.

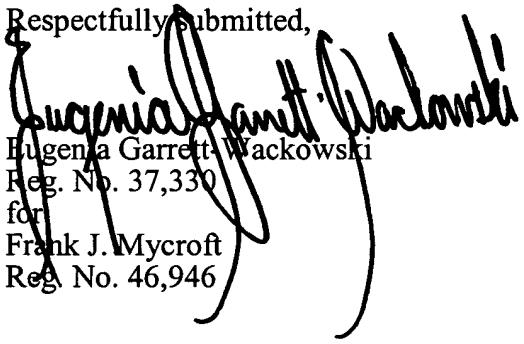
Neither the Jensen nor the Gallina references address the above deficiencies of the combined references. In view of the above, the Applicants respectfully request that the above grounds for rejection be reconsidered and withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



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'Reversible chemical sphincterotomy' by local application of glyceryl trinitrate

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Nitric oxide has emerged as one of the most important neurotransmitters mediating internal anal sphincter relaxation. The effect of glyceryl trinitrate, a nitric oxide donor, on anal tone was examined. Maximum resting pressure, predominantly a function of the smooth muscle internal anal sphincter, was measured before and 20 min after application of 0·2 per cent glyceryl trinitrate ointment in ten patients. Pressure decreased by a mean of 27 per cent (95 per cent confidence interval 18–36 per cent) ($P = 0·0004$) after administration of the drug. A further 20 patients were then randomized to either active or placebo ointment. Anal pressure was significantly decreased ($P = 0·002$) in those who received 0·2 per cent glyceryl trinitrate, but there was no significant reduction in the control patients. Mild headache occurred in two patients who were given the active preparation and in one who received placebo. Manometry was repeated 9 h after application of glyceryl trinitrate and showed a sustained decrease in pressure in two patients. Topical glyceryl trinitrate may have a role in the treatment of anal fissure, haemorrhoids, certain types of constipation and anal pain. It may also reduce injury to the internal sphincter during peranal operations.

Several anorectal disorders are associated with increased resting anal pressure[1]. Raised resting pressure is important in the pathogenesis of anal fissure, possibly by impairing tissue perfusion[2–4]; surgical reduction of resting pressure is the recommended treatment for chronic anal fissure[5]. It is less certain, however, that increased anal pressure is the cause of haemorrhoids, but reduction of resting tone surgically may effectively relieve symptoms[6,7].

Although increased resting tone is not usually a feature in patients suffering from anal pain[8] or constipation[9], there are subgroups in which surgical reduction of pressure results in clinical improvement[10]. Finally, sphincterotomy[11] and anal dilatation[12] may decrease pain after haemorrhoidectomy.

Surgical operations to reduce anal pressure are effective but carry a significant risk of permanent minor impairment of continence[13,14]. Internal sphincterotomy is less likely to result in major incontinence than anal dilatation, but incontinence to flatus or mucus is common after both procedures[14,15]. The internal anal sphincter is responsible for the majority of resting anal pressure[16] and may be relaxed by pharmacological agents; this may permit effective treatment without the risk of permanent incontinence if unpleasant systemic side-effects, a short duration of action or the need for intravenous administration do not limit clinical application[17–23].

Recent *in vitro* and *in vivo* studies in animals have established that nitric oxide is probably the most important inhibitory neurotransmitter in the internal anal sphincter[24–26]. Glyceryl trinitrate is a readily available nitric oxide donor used predominantly in the treatment of coronary artery disease. The drug is available as an ointment for transdermal systemic administration. In the present study the ability of this agent to reduce resting anal pressure was examined.

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Patients and methods

Patients presenting for physiological assessment of anal disorders were studied. The study comprised three parts: an initial investigation to establish the acceptable drug concentration (two patients), a pilot study of ten patients and a randomized placebo-controlled trial of twice that number.

Test preparations

The test ointments were made in the hospital pharmacy. Aliquots were prepared of undiluted Percutol (2 per cent glyceryl trinitrate ointment: Cusi Laboratories, Haslemere, UK) and of Percutol diluted 1:2, 1:4 and 1:10 in yellow soft paraffin to give final glyceryl trinitrate concentrations of 1, 0·5 and 0·2 per cent. These preparations were tested on sequential days in two patients with recording of all side-effects.

For subsequent studies 0·2 per cent glyceryl trinitrate was used as the active preparation. The placebo application consisted of yellow soft paraffin alone for the controlled trial. Both preparations were placed in small containers and labelled by number and date of preparation. Only the pharmacist was aware of the contents of each container. Preparations were discarded 4 weeks after formulation because of possible instability and bacteriological contamination.

Liberal digital application of the preparation was made externally to the anus. Care was taken to avoid passing the finger into the anal canal so as to prevent any possible effect on anal pressure as a result of dilatation.

Manometry

Manometry was performed before and 20 min after application of the preparation being tested. Maximum resting pressure in the anal canal was measured using a water-filled microballoon system, applying a station pull-through technique. Pressure was displayed graphically on a chart recorder. The microballoon was inserted into the rectum without prior digital or proctoscopic examination and withdrawn in 0·5-cm increments. Sufficient time was allowed for pressure to stabilize at each station. The pressure at each station was taken as the minimum value recorded at that level of the anal canal during the recording period. The highest such value was recorded as the maximum resting pressure.

Side-effects

All subjects in the controlled study were specifically asked whether they had a headache or any other unexpected symptom after completion of the manometric measurements.

Statistical analysis

Analysis was performed with the Minitab release 8 statistical package (Minitab; State College, Philadelphia, Pennsylvania, USA). Normality of distributions was confirmed using correlation with normally transformed data. Pressure before and after application of the preparation was analysed employing Student's paired *t* test. The treatment and placebo groups were compared by Student's unpaired *t* test.

Results

Effects of different preparations

The 2, 1 and 0·5 per cent glyceryl trinitrate preparations caused headache in both patients in the initial study. These lasted several hours and prevented the patients from resuming work for the remainder of the day. Application of the 0·2 per cent preparation was followed by a mild headache lasting 30 min in one of the patients; the other reported no ill effects. The effect of 0·2 per cent glyceryl trinitrate was thus examined in the remaining studies.

Pilot study

Patients. Seven men and three women of mean(s.d.) age 39(13) (range 19–67) years were studied. There was a broad spectrum of diagnoses: there were two normal subjects, two with pruritus ani, four with haemorrhoids, one with anal fissure and one with anal pain.

Manometry. Maximum resting pressure decreased in all patients (*Fig. 1*) from a mean value of 99 (95 per cent confidence interval (c.i.) 74–124) cmH_2O to 73 (95 per cent c.i. 50–96) cmH_2O ($P = 0{\cdot}0004$). This represented a mean reduction of 27 per cent (95 per cent c.i. 18–36 per cent). No patients had sustained ultraslow wave activity. The drop in pressure was sustained in two patients in whom manometry was repeated 4 and 9 h after drug application (*Fig. 2*).

Controlled trial

Patients. Details of the 20 patients are given in *Table 1*. The control and treated patients were similar with respect to age, sex ratio, diagnosis and initial maximum resting pressure.

Manometry. In patients who received the active preparation the maximum resting pressure decreased from a

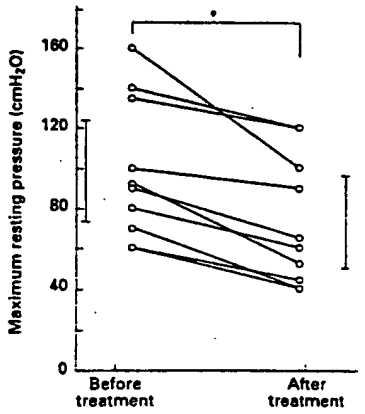

Fig. 1 Maximum resting pressure before and 20 min after topical application of 0·2 per cent glyceryl trinitrate ointment. Bars are 95 per cent confidence intervals. *$P = 0{\cdot}0004$ (Student's *t* test)

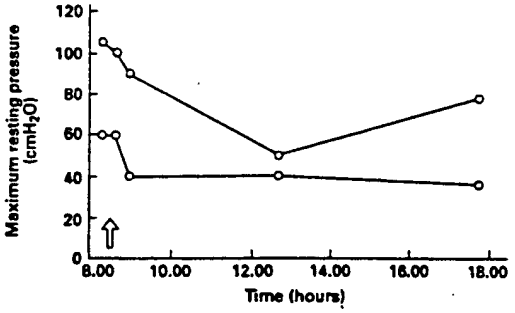

Fig. 2 Repeated measurement of maximum resting pressure in two patients. Topical 0·2 per cent glyceryl trinitrate ointment was applied at 08.40 hours (arrow) after the second measurement. Pressure remained depressed on each of three subsequent measurements up to 9 h after the application

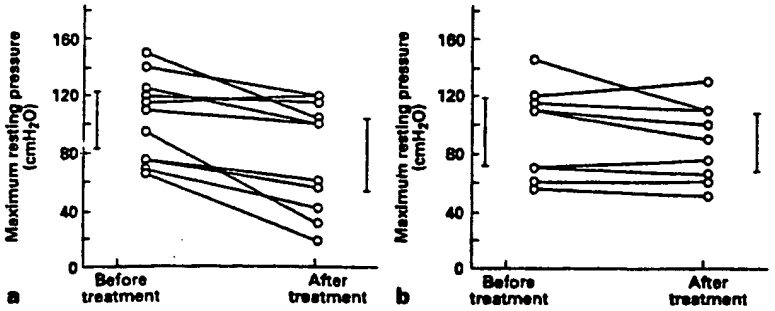

Fig. 3 Maximum resting pressure before and 20 min after application of a 0·2 per cent glyceryl trinitrate ointment and b placebo. Bars are 95 per cent confidence intervals. $P = 0{\cdot}0024$ (pressure before *versus* pressure after application of glyceryl trinitrate), $P = 0{\cdot}033$ (change in pressure after application of glyceryl trinitrate *versus* change in placebo group); Student's *t* test

Table 1 Clinical features of patients who received 0·2 per cent glyceryl trinitrate and placebo

	Glyceryl trinitrate	Placebo
No. of patients	11	9
Age (years)*	48·9 (32·3–65·5)	48·7 (39·6–57·8)
Sex ratio (M:F)	6:5	6:3
Initial maximum resting pressure (cmH_2O)*	103 (83–123)	95 (71–119)
Diagnosis		
Constipation	4	2
Soiling	3	3
Haemorrhoids	3	3
Rectal prolapse	0	1
Perianal pain	1	0

*Values are mean (95 per cent confidence interval)

mean of 103 (95 per cent c.i. 83–123) cmH_2O to 79 (95 per cent c.i. 53–104) cmH_2O ($P = 0·0024$). The mean initial resting pressure was 95 (95 per cent c.i. 71–119) cmH_2O in control subjects. The mean pressure after application of the placebo (88 (95 per cent c.i. 67–108) cmH_2O) was not significantly different (*Fig. 3*). The difference between those who received 0·2 per cent glyceryl trinitrate and the control patients was significant ($P = 0·033$). Sustained ultraslow wave activity was not observed.

Side-effects. On direct questioning two patients who received glyceryl trinitrate reported headache as did one of the controls. In each case the headache was mild and was not volunteered before questioning. No other symptoms were described.

Discussion

In vitro animal[24] and human[25] experiments and *in vivo* animal studies[26] have now established nitric oxide as probably the most important inhibitory neurotransmitter of the internal anal sphincter. Although several other substances[17–23] decrease anal pressure, none has found clinical application. The discovery of the nitric oxide pathway presents a valuable new avenue for manipulation of anal pressure. Glyceryl trinitrate is one of a group of organic nitrates that binds to protein receptors, releasing nitric oxide[27]. In its most common clinical role as a treatment for angina pectoris, systemic absorption results in generalized vasodilatation. Local activity after topical application has also been successful in the treatment of Raynaud's disease[28], impotence[29] and extravasation injuries[30], and as an aid to venepuncture[31].

The present study demonstrates that a dilute preparation of 0·2 per cent glyceryl trinitrate effectively reduces anal tone without the intolerable side-effects associated with more concentrated preparations. Although only two patients have undergone repeated manometry, it is apparent that the agent has a prolonged duration of action. Clinically, this means that adequate efficacy will not require application more frequently than twice daily; more detailed studies are necessary to establish the duration of action more precisely. In this study the preparation was applied externally to avoid any effect of digital dilatation on subsequent pressure recordings. This restriction would not apply in clinical use and it is possible that internal application may result in a greater effect or a longer duration of action.

Several important potential clinical applications are apparent. It is possible that glyceryl trinitrate may assist anal healing by reducing anal pressure and inducing local vasodilatation; this is a role particularly suited to the treatment of anal fissure, where there is impaired vascular perfusion[4]. The reduction in anal pressure of 27 per cent is comparable to the 26–50 per cent reduction reported after surgical sphincterotomy[1,3,32–34].

It is yet to be established whether the preparation used will be effective in the treatment of anal fissure: only one patient with fissure was included in this preliminary study and the mechanism of raised pressure in patients with this lesion remains obscure. In addition to increased resting anal pressure, ambulatory manometry reveals a marked reduction in both the number and extent of spontaneous internal sphincter relaxations in patients with anal fissure[35], supporting the concept that the sphincters are involved in the primary pathological process.

Pharmacological reduction of anal tone may also be a useful adjunct to surgical treatment. When haemorrhoidectomy is necessary, the reduction of anal tone to reduce postoperative pain may be preferable to concomitant sphincterotomy or anal dilatation. Intraoperative relaxation of the internal sphincter may also prevent injury during operation on the anorectum. Anal dilatation that occurs during endoanal anastomosis may contribute to the reduction in resting pressure following restorative proctocolectomy and coloanal anastomosis[36–38].

Pharmacological manipulation of nitric oxide-mediated relaxation of the anal sphincter presents a new means of treating anorectal dysfunction and a novel research tool into basic pathophysiological mechanisms.

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Topical glyceryl trinitrate in the treatment of chronic anal fissure

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The aetiology of anal fissure is unclear, but there is an association with high maximum resting pressure (MRP). Internal sphincterotomy reduces MRP and heals fissure through an increase in local blood supply. Glyceryl trinitrate (GTN) is a nitric oxide donor which contributes to internal anal sphincter relaxation via a non-adrenergic non-cholinergic pathway. GTN ointment was applied topically in different concentrations to the anal margin in patients with chronic anal fissure to monitor its effect primarily on MRP and secondarily on fissure healing. Nineteen patients with chronic anal fissure were treated with ointment containing increasing concentrations of GTN (0·2–0·8 per cent) to produce a reduction in MRP of greater than 25 per cent. The actual dose of GTN varied as no standard delivery system has been developed, but a 'typical amount' of GTN ointment weighed about 200 mg. In 15 of 19 patients, a concentration greater than 0·2 per cent was required to lower the MRP by at least 25 per cent. The minimum concentration of GTN that reduced the resting pressure by at least 25 per cent was prescribed and local application was carried out by the patient twice daily for 6 weeks. At 6 weeks, nine patients had healed, six required sphincterotomy and four were lost to follow-up. Eight of the nine patients with healed fistula required a GTN concentration of 0·3 per cent or more. Sixteen patients were resistant to the usually effective dose of 0·2 per cent GTN. In three there was tachyphylaxis and the duration of action of GTN was less than the 12 h described previously in control patients. Two patients did not fulfil the study because of headache.

The aetiology of anal fissure is unknown but patients tend to have a high anal maximum resting pressure (MRP)[1–4] which if reduced leads to fissure healing[2] and increased blood flow to the fissure ulcer[5]. Anal dilatation and lateral internal sphincterotomy lower the MRP and heal the fissure in most cases but with the complication of minor incontinence in up to 30 per cent of cases[1,3,6–10].

There is evidence that nitric oxide is involved as the inhibitory neurotransmitter to the human internal anal sphincter[11,12]. Glyceryl trinitrate (GTN) is an organic nitrate and donates nitric oxide. Topical GTN ointment has been shown to lower the MRP in asymptomatic controls and in patients with a variety of anal conditions[13]. Nitric oxide binds to the Fe^{2+} moiety of the heme molecule of guanylate cyclase in the smooth muscle cell cytoplasm and increases levels of the secondary messenger guanosine 3′,5′-cyclic monophosphate (cGMP)[14]. It is possible that nitric oxide produces relaxation of the smooth muscle cell via an alteration to potassium-activated channels in the cell membrane producing hyperpolarization; this may be through at least one route that involves cGMP[15]. Although other drugs are known to be direct or indirect nitric oxide donors, GTN was used as it is readily available, is absorbed transcutaneously and has been extensively investigated in this centre. Headaches are a common side-effect of GTN use; syncope and other side-effects are also possible[16].

Previous experience from this centre in control patients has shown that the reduction in MRP is 27 per cent and side-effects are minimized when 0·2 per cent GTN is applied to the anal margin[13]. An initial study by Kennedy *et al.*[17] using topical GTN to lower MRP in patients with anal fissure has, however, shown a healing rate only marginally better than that after conservative treatment.

Gorfine[18] used GTN in a heterogenous group of 15 patients with anal 'ulcers' and fissures of 2 days' to 2 years' duration. A dose of 500–1000 mg of 0·5 per cent topical GTN relieved pain but there was no indication as to why this dose was selected and anal manometry was not performed. The author concluded that '...it seems highly probable that the pain relief... was caused by nitric oxide-mediated relaxation of the IAS [internal anal sphincter]'.

There are three possible reasons for the failure of topical GTN to produce healing of a chronic anal fissure: a raised threshold to the relaxant action of topical GTN on the internal anal sphincter in patients with fissure compared with asymptomatic controls, a shorter than expected duration of action, and the development of tachyphylaxis (a previously effective dose becoming ineffective with time).

This study aimed to find an appropriate concentration of GTN that lowered MRP in the individual patient with fissure; and to determine its effectiveness in curing anal fissure.

Patients and methods

Outpatients presenting with chronic anal fissure were included in the study. Consecutive patients were accrued by a single researcher who was not blinded. Patients with Crohn's disease, women who might have been pregnant or who were considering pregnancy, and patients with ischaemic heart disease were excluded. Anal manometry was performed before and during treatment with GTN ointment. Ethical permission was obtained from the district ethics committee.

Definition of chronic anal fissure

Chronic anal fissure was taken to be an anal fissure in an outpatient who gave a history of anal pain on defaecation for at least 2 months. Some also experienced bleeding on defaecation, others a sentinel tag and/or an anal papilla, but these signs or symptoms were not considered necessary for inclusion in the study.

Glyceryl trinitrate

Preparation. Test ointments were prepared in the hospital pharmacy. Percutol (2 per cent GTN ointment; Cusi, Haslemere,

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UK) was diluted in yellow soft paraffin to produce preparations of 0·2, 0·3, 0·4, 0·6 and 0·8 per cent GTN. Yellow soft paraffin is compatible with 2 per cent Percutol and was therefore used as the agent for dilution. Preparations were kept in a cool place in an opaque glass container with a screw top lid and discarded 4 weeks after formulation.

Dose. Like all other reported studies of the use of GTN[2,17,18] or isosorbide dinitrate[19] to treat anal conditions, there is no standard dose delivery system currently available. As with use of other ointments in the treatment of anal conditions, the amount used is variable, although in this study all patients appeared to follow instructions to use an amount that could be readily rubbed into the anal margin without creating a messy excess. This 'typical dose' was about 200 mg.

The amount of GTN absorbed was not measured but the paucity of side-effects of headache indicated minimal systemic absorption.

Assessment of healing

Patients were asked about the presence or absence of pain and bleeding on defaecation at each visit. Following surgical sphincterotomy, pain disappears before complete healing of the fissure, which may take several weeks. Therefore if there was no pain or bleeding on defaecation and there was evidence of complete or partial healing of the fissure with re-epithelialization, the treatment was deemed a success. However, this study was not primarily aimed at healing the fissure but at assessing the relative resistance of patients with fissure to the effect of GTN on MRP.

First visit

Anal manometry was performed using a 4-mm water-filled microballoon connected to a transducer and chart recorder. The catheter was inserted with a lubricant gel containing chlorhexidine but without local anaesthetic. After careful and thorough explanation of the test, all patients tolerated the microballoon well.

MRP was identified using a station pull-through technique and the catheter taped to the buttock leaving the balloon in the anal canal at that level. A steady resting pressure was allowed to develop over the next 12 min and its value recorded. Previous experience has shown that 12 min is sufficient for patients to attain a steady MRP.

GTN ointment (0·2 per cent) was applied circumferentially to the anal margin and gently rubbed in a clockwise direction for 5–8 s with the gloved index finger of the researcher. The ointment was not applied intra-anally. After a further 12 min, if a steady state had been reached, the MRP was recorded. If the pressure had not fallen by 25 per cent and the patient had not developed any side-effects, excess ointment was wiped away from the anal margin and the next concentration of GTN applied.

The same period of stabilization was permitted at each concentration and the procedure was repeated up to a concentration of 0·8 per cent GTN or until the resting pressure had fallen by 25 per cent of the original value. The minimum concentration of GTN ointment that lowered the MRP by at least 25 per cent was prescribed and the patient was instructed to use the ointment twice a day for 6 weeks. The patient was then reviewed at the second visit after 3 weeks.

Patients were questioned about compliance at each visit but no formal diary was maintained.

Second visit

Patients were asked to use the ointment on the morning of the test. Manometry was performed as at the first visit. The MRP after a 12-min stabilization period and the response of the MRP to a repeated application of the concentration of GTN ointment originally prescribed after 12 min were recorded. If this concentration now failed to lower the MRP, higher concentrations were applied. The development of tolerance to GTN (tachyphylaxis) and the duration of action of GTN could be inferred from these MRP measurements. Patients were specifically asked about headaches during treatment. They were then asked to apply GTN ointment for a further 3 weeks.

Final visit

At the end of 6 weeks the patient returned to the referring clinician for assessment of fissure healing.

Results

An overview of the results is given in *Fig. 1.*

Patient characteristics

The mean age was 44 (median 41, range 27–76) years; there were 13 men and six women. All had a posterior fissure. One woman subsequently developed an anterior fissure during treatment.

Pretreatment anal manometry

The mean MRP in 13 men was 155 (median 140, range 100–280) cmH_2O and that in six women 120 (median 123, range 70–170) cmH_2O.

First visit

MRP after topical application of 0·2 per cent GTN ointment in patients with anal fissure is shown in *Fig. 2.* There was a significant overall reduction ($P = 0·007$, Wilcoxon paired test) but this resulted from large reductions in a few patients. A reduction of 25 per cent or more occurred in only three of the 19 patients. One patient developed headache despite a fall in MRP of only 16 per cent and withdrew from the study.

Of the remaining 15, a fall of 25 per cent was eventually achieved at a higher concentration in all but one who did not respond to 0·8 per cent GTN. He was referred immediately for sphincterotomy. Another patient did not return for subsequent visits. Sixteen patients therefore remained in the study.

Second visit

Of the 16 patients, 15 returned for the second visit. Six were symptom-free and the fissure had healed with ointment containing 0·2 per cent (one patient), 0·3 per cent (four) and 0·4 per cent (one) GTN. Nine still had a symptomatic fissure. Of these, two had stopped treatment and were referred for sphincterotomy after declining repeat anal manometry. The remaining seven had repeat manometry performed less than 8 h after application of GTN ointment (*Fig. 3*).

The percentage fall in MRP during the first and second visits of these seven patients is given in *Table 1.* Patients 1 and 2 showed evidence of tachyphylaxis and the previously effective dose of GTN ointment produced no reduction in MRP. The results for patients 3 and 4 suggest a duration of action of the GTN ointment of less than 8 h. Repeated application of GTN ointment reduced the MRP by 50 and 83 per cent respectively. In the remaining four patients, there was evidence of both tachyphylaxis and short duration of action.

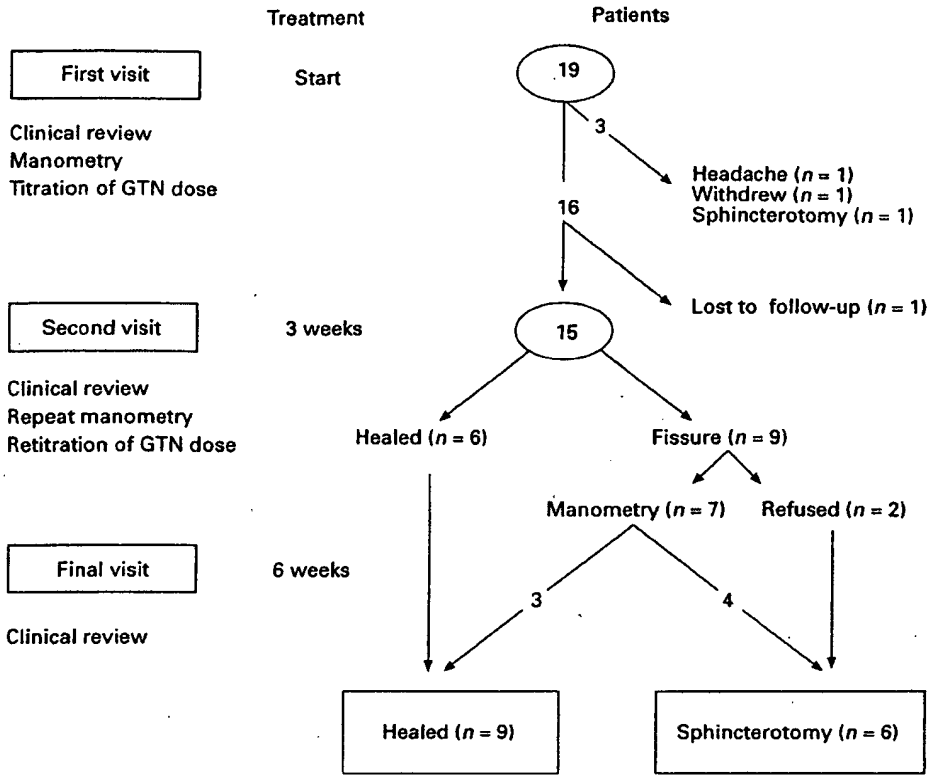

Fig. 1 Flow chart of glyceryl trinitrate (GTN) treatment in 19 patients over 6 weeks

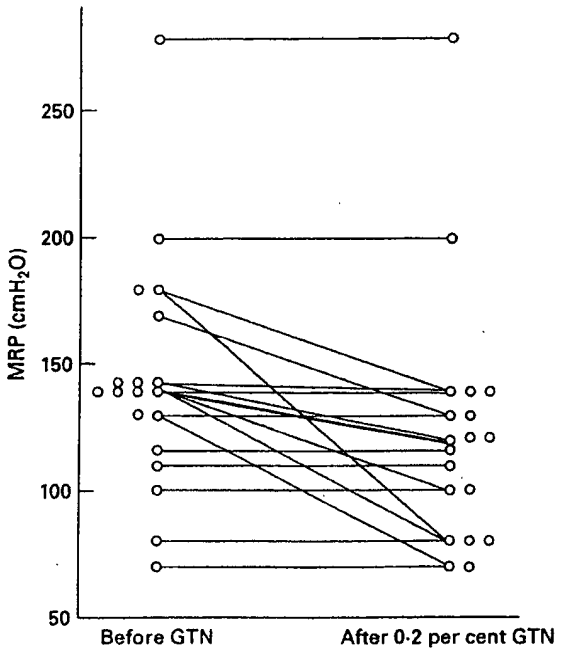

Fig. 2 Maximum resting pressure (MRP) in 19 patients before and after application of 0·2 per cent glyceryl trinitrate (GTN) ointment

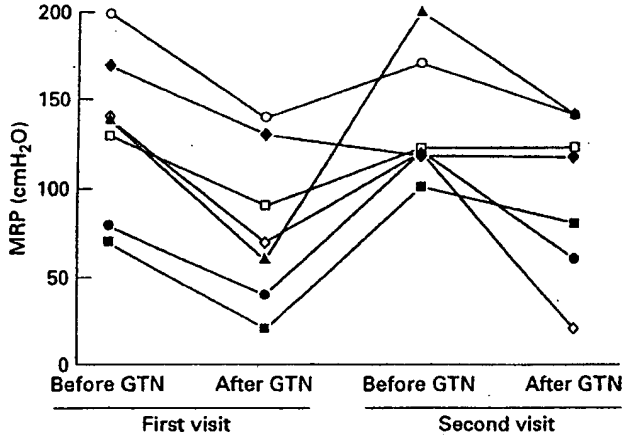

Fig. 3 Effect of glyceryl trinitrate (GTN) on maximum resting pressure (MRP) at the first and second visit in seven patients with persisting fissure. In these seven patients there was no significant difference between the MRP after GTN administration at the first and follow-up manometry assessments ($P = 0{\cdot}25$, Wilcoxon paired test)

Final visit

The fissure had healed in nine of the 13 patients remaining in the study. Four patients without fissure healing were referred for sphincterotomy. Of the nine patients whose fissure healed with GTN treatment, only

Table 1 Tachyphylaxis and duration of action of glyceryl trinitrate ointment as the possible cause of failure to heal seven patients

Patient no.	Percentage reduction in MRP after GTN ointment		Possible cause of failure
	First visit	Second visit	
1	25	0	Tachyphylaxis
2	28	0	Tachyphylaxis
3	50	50	Duration
4	50	83	Duration
5	71	20	Tachyphylaxis/duration
6	30	18	Tachyphylaxis/duration
7	57	30	Tachyphylaxis/duration

GTN, glyceryl trinitrate; MRP, maximum resting pressure

one healed with 0·2 per cent GTN ointment while five required 0·3 per cent, one 0·4 per cent and two 0·8 per cent GTN.

It was not possible to predict healing from the initial manometric response to GTN ointment.

Discussion

Surgical sphincterotomy is an excellent procedure for the long-term cure of chronic anal fissure. It probably works by reducing resting anal pressure (by as much as 50 per cent)[4,20–22]. There is evidence from laser Doppler studies that this may lead to improved perfusion of the pecten which might then facilitate healing of what are now known to be relatively ischaemic ulcers[1,23]. Further studies are required to confirm this.

As most fissures come and go an initial non-operative approach has been advocated by some[24,25]. Topical GTN, by producing a reversible 'chemical sphincterotomy', might increase the success of medical treatment. In a single-blind placebo-controlled trial Loder *et al.*[13] showed that 0·2 per cent GTN applied to the anal margin of patients with a variety of anal disorders reduced mean resting pressure by an average of 27 per cent. Another single-blind randomized placebo-controlled study in 42 patients with anal fissure showed a mean reduction in resting pressure of only 14 per cent but healing in 46 per cent within several weeks[17]. A further study that reported almost universal success with 0·5 per cent GTN did not use anal manometry to show the effects of this agent on MRP[18].

The present study had an overall success rate of 47 per cent (nine of 19 patients), compatible with results of others using GTN to lower MRP in patients with fissure[17]. It has also demonstrated some possible reasons for the high failure rate. First, the patients in the present study were relatively resistant to topical GTN. Only three showed a significant fall in MRP (more than 25 per cent) at a dose of 0·2 per cent GTN. This was a much lower proportion than that reported by Loder *et al.*[13]. In the latter study only one of 30 patients had an anal fissure. It is possible, therefore, that the pathogenesis of anal fissure is related in part to an insensitivity of the internal sphincter to endogenous nitric oxide.

Second, the duration of action of GTN ointment was less than the 12 h reported in two control patients[13]. The actual duration of action could not be determined in the present study but appeared to be less than 8 h in five of the seven patients in whom the fissure failed to heal. Further work on the dose–response relationship and the duration of action of GTN ointment is required. Where patients received more than one dose of different concentrations of GTN at a single session, it is possible that they effectively received a cumulative dose of GTN. However, it would not have been practical to ask the patients to return the following day if they failed at a particular concentration. In some cases, this may have meant patients returning on several consecutive days.

Third, five patients demonstrated an acquired tolerance to GTN. Tachyphylaxis is well recognized by physicians using nitric oxide donors for angina pectoris[26]. Tolerance is thought to result from a depletion of sulfhydryl groups which are necessary for biotransformation of organic nitrates to nitric oxide[27,28]. It may be possible to treat anal fissure with nitric oxide donors that do not rely on sulfhydryl groups and therefore are not subject to tachyphylaxis.

Before conducting a meaningful randomized trial of GTN *versus* placebo in patients with fissure, knowledge of the effective dose of GTN, appropriate frequency and duration of application and dose increments necessary to counter the effects of tachyphylaxis is required. Until these difficulties with GTN are overcome, the ad hoc use of an arbitrary concentration of GTN in an outpatients department without the use of manometry has little to recommend it.

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